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Carotenoid-based nestling colouration and parental favouritism in the great tit

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Abstract While elaborate carotenoid-based traits in adult birds may have evolved as honest signals of individual quality in the context of sexual selection or other social interactions, the function of carotenoid-based colours in juveniles is less well understood. We investigated the hypothesis that carotenoid-based nestling colouration has evolved in response to parental preference of intensely coloured offspring during food provisioning. In a field experiment, we manipulated nestling plumage colouration by a carotenoid-supplementation and analysed the parental food provisioning behaviour before feather appearance and at the end of the nestling stage. Carotenoids per se did not influence the nestling's begging behaviour or parental feeding decisions and we found no evidence that carotenoid-based colouration in nestling great tits has a signalling function in parent-offspring interactions. Parents did not discriminate between intensely coloured and control offspring in their food provisioning and in accordance with this finding intensely coloured nestlings were not heavier or larger at the end of the nestling stage. Alternative explanations for the evolution of carotenoid-based colours in nestling birds are discussed.

Keywords Begging · Feeding behaviour · Food provisioning · Parent-offspring interaction · Signalling

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Introduction

Conspicuous carotenoid-based colours are displayed in numerous animal species and have an important role in communicating an individual's quality to potential rivals or mates (reviewed in Andersson 1994; Hill 1999). Consequently, carotenoid-based ornaments are found mainly in adults. Nestling plumage tends to lack carotenoids and shows more often pigmentation by melanins (Brush 1978). It has been suggested that melanin-based colouration is more cryptic and thus of high selective advantages for juveniles in the nest or after fledging (e.g. Booth 1990). There are, however, a few bird species where a conspicuous carotenoid-based colouration is expressed already in the nestling plumage, but information about its functional significance is scant. Nestlings moult the body plumage before the first breeding attempt (post-juvenile moult, Jenni and Winkler 1994) and at least in great tits (*Parus major*) nestling plumage colour is not significantly related to colouration as a first year breeder (Fitze et al. 2003). It is thus unlikely that carotenoid-based colours in nestlings evolved via sexual selection.

Carotenoids are important for the regulation of immune function and the prevention of oxidative stress (Bendich 1989; Edge et al. 1997; Olson and Owens 1998; Chew and Park 2004). Parasite-resistant nestlings might divert carotenoids from immune function to plumage pigmentation and thereby signal parasite resistance to parents. It has thus been hypothesised that nestling ornamentation evolved via parental preference for nestlings that signal their higher quality by carotenoid-based plumage colour (i.e. Saino et al. 2000).

Empirical evidence for parental preference of colour ornaments during food provisioning was found in a study on American coots (*Fulica americana*) (Lyon et al. 1994). Nestlings of this species show conspicuous orange and red ornaments on the head (Lyon et al. 1994) and trimming of these ornaments led to a lower feeding and growth rate compared to the ornamented siblings. At

least three reasons might explain why parents preferentially feed brightly coloured young: (1) parents may selectively invest into high-quality offspring (see above), (2) colourful nestlings may be favoured because of a parental colour preference that has evolved in another context (sensory exploitation, Lyon et al. 1994; Ryan 1998), or (3) ornamented young may be easier to detect (Ficken 1965, but see Kilner and Davies 1998 for nestling mouth colour).

To investigate the function of carotenoid-based nestling plumage, we performed a field experiment on great tits (*P. major*). The great tit is a small hole-nesting passerine that develops a carotenoid-based colouration early in life. Its yellow breast plumage colouration results from the carotenoids lutein and zeaxanthin that are ingested with the food and deposited unmodified in the developing feathers (Partali et al. 1987). Earlier studies showed that the carotenoid-based colour expression in nestling great tits is variable among individuals. It depends on the amount of carotenoids ingested with the food, the nestling's condition, and the nestling's genetic background (Fitze et al. 2003; Tschirren et al. 2003) and thus, honestly signals nestling quality. However, its functional significance is unknown so far. Here, we tested the hypothesis that the conspicuous yellow plumage colouration of nestling great tits has a signalling function in parent-offspring interactions during feeding. We manipulated the nestling plumage colouration using a carotenoid-supplementation experiment and analysed parental food provisioning to intensely coloured and control nestlings. To distinguish between parental preference for intense plumage colouration and potential effects of the carotenoids per se on sibling interactions or begging (e.g. Saino et al. 2000; Hunt et al. 2003) that might indirectly influence parental food provisioning, we filmed the parental feeding behaviour before feather appearance and at the end of the nestling stage (Christe et al. 1996). If intense nestling plumage colouration is favoured by parents, we predict that nestlings of the carotenoid-supplemented group receive more food after plumage appearance only, but not before feather growth. Conversely, if carotenoids affect the sibling interactions or the begging of nestlings, differences in parental food provisioning between carotenoid-supplemented and control nestling should be found already before feathers are visible.

Materials and methods

General experimental procedure

The experiment was performed in 1999 in a great tit population breeding in nest boxes in the Forst, a forest near Bern, Switzerland (46°54′N 7°17′E/46°57′N 7°21′E). Nest boxes were regularly visited from the beginning of the breeding season onwards to determine the start of egg laying and the hatching date.

Nestling body mass was measured 1, 7 and 15 days post-hatching using an electronic balance with a precision of 0.01 g. On day 15, we measured the length of the metatarsus to the nearest 0.1 mm using a calliper. Nestlings were marked individually by clipping down feathers 1 day post-hatching and were ringed with aluminium rings 7 days post-hatching.

Manipulation of plumage colouration

The plumage colouration was experimentally manipulated by supplementing half of the nestlings within each nest with the carotenoids lutein and zeaxanthin (Tschirren et al. 2003). Nestlings were ranked according to body mass within each nest ($n = 43$ broods) 1 day post-hatching. The heaviest nestling was randomly assigned to be carotenoid-supplemented or to receive placebos as a control. Feeding treatment was alternated through the mass-based rank list within the nest. Starting 3 days post-hatching, the nestlings were fed every other day for a total of six times. Nestlings of the carotenoid-supplemented group were fed with 17 mg ($\pm$ 0.25 mg) carotenoid beadlets per feeding containing 5.58% lutein and 0.44% zeaxanthin (Hoffmann, La Roche, Basel, Switzerland), while nestlings of the control group were fed with 17 mg ($\pm$ 0.25 mg) placebo beadlets (Hoffmann, La Roche) (Tschirren et al. 2003). The lutein/zeaxanthin ratio of the carotenoid beadlets was similar to the ratio found in the natural diet of great tit nestlings (Partali et al. 1987). One day post-hatching, mean body mass of carotenoid and placebo fed nestlings did not significantly differ (mean body mass of carotenoid-supplemented nestlings: 2.52 ± 0.05, placebo-fed nestlings: 2.49 ± 0.06 g, paired t-test: $t_{1,42} = 1.293$, $P = 0.203$).

Fifteen days post-hatching the nestlings were photographed with a digital camera under standardised conditions as described in Fitze and Richner (2002) and Tschirren et al. (2003) to assess colour differences between the treatment groups. Hue (H), saturation (S) and brightness (B) of the birds' plumage colouration was calculated (Endler 1990). Using principal component analysis, the first principal component of the colour parameters HSB was derived and taken as an overall measure of the plumage colouration (hereafter referred to as colour PC1). Colour PC1 explained 53.75% of the total variance (Eigenvectors: $H = -0.627$, $S = 0.681$, $B = 0.378$; PC2 explained 30.87%). In two nests plumage colouration could not be measured due to technical problems with the camera.

Parental food provisioning

The feeding behaviour of the adult birds was analysed to investigate the potential signalling function of the carotenoid-based nestling colouration in parent-offspring interactions. To detect potential effects of the carotenoids per se on sibling interactions or begging behaviour that might indirectly influence parental food

provisioning, we filmed the broods on day 7 post-hatching when the nestling's plumage is not yet developed. The broods were filmed a second time on day 14 post-hatching to investigate parental preference for more intensely coloured nestlings.

Before filming nestlings were marked with red paint on the head for identification (Kölliker et al. 1998). Nestlings of a treatment group within a nest received the same mark and marks were alternated between treatment groups. The broods were filmed in the nest box with a video camera sensitive to infrared as described in Christe et al. (1996). For the analyses of the videotapes, the first five feeding visits were discarded to avoid a bias due to disturbance during installation of the camera. Food provisioning rates of the male and female parent, the group of young fed and prey size were then recorded during the following hour. Prey size was classified as small, intermediate or large (Kölliker et al. 1998). The food quantity delivered to the nestlings was calculated as the product of feedings per hour per nestling and mean prey size.

Seven days post-hatching, the begging intensity of carotenoid-supplemented and control nestlings was analysed in a random sub-sample of broods ($n = 26$). Begging intensity (posture) of the nestlings was scored as 0 = calm, 1 = weak gaping, 2 = persistent gaping, 3 = gaping, neck fully stretched and 4 = gaping, neck fully stretched, wings flapping (Kölliker et al. 1998).

The parental feeding behaviour and nestling begging intensity were analysed blindly with respect to the carotenoid treatment of the nestlings. Sample size in the analysis of male and female food provisioning differs as in some nests the female or male parent did not feed the nestlings during filming. The results of the analyses (see below) do not change if the nests where only one parent fed during filming were excluded. The video recordings of six broods on day 7 could not be analysed due to technical problems during filming.

Statistical analyses

Differences between carotenoid-supplemented and placebo fed nestlings were analysed by paired *t*-tests. Begging intensity was analysed by Wilcoxon signed-rank test for paired data. All tests were two-tailed with a significance level set at $P \leq 0.05$. Means ± SE are given. Statistical analyses were performed using JMP IN 4.0 (Sall and Lehmann 1996). Effect sizes (*d*) corrected for the correlation between dependent values are calculated according to Cohen (1988).

Results

Food provisioning and begging before feather appearance

Feeding rate and food quantity delivered to carotenoid-supplemented or placebo fed young was not significantly different before feather appearance (male: feeding rate: $t_{1,33} = -0.881$, $P = 0.385$, food quantity: $t_{1,33} = -0.313$, $P = 0.756$, Fig. 1; female: feeding rate: $t_{1,35} = -0.394$, $P = 0.696$, food quantity: $t_{1,35} = -0.342$, $P = 0.735$, Fig. 1; total parents: feeding rate: $t_{1,36} = -0.939$, $P = 0.354$, food quantity: $t_{1,36} = -0.505$, $P = 0.617$). Carotenoid-supplemented and placebo fed young did further not beg at significantly different intensities ($W = -9.0$, $P = 0.762$, $n = 26$) and there were no differences in body mass between treatment groups (carotenoid-supplemented: 11.54 ± 0.134 g, placebo: 11.41 ± 0.139 g; $t_{1,42} = 0.737$, $P = 0.465$). Thus, we found no indication that carotenoids per se influenced sibling interactions or begging and thereby parental feeding decisions.

Plumage colouration

Plumage colouration 15 days post-hatching differed significantly between carotenoid-supplemented and control nestlings in hue (carotenoid-supplemented: 42.02° ± 0.055, placebo: 42.52° ± 0.059, $t_{1,40} = -7.338$, $P < 0.0001$), saturation (carotenoid-supplemented: 0.541 ± 0.003, placebo: 0.488 ± 0.003, $t_{1,40} = 11.165$, $P < 0.0001$), and colour PC1 (carotenoid-supplemented: 0.602 ± 0.092, placebo: −0.594 ± 0.096, $t_{1,40} = 9.886$, $P < 0.0001$), but not in brightness (carotenoid-supplemented: 0.816 ± 0.003, placebo: 0.821 ± 0.003, $t_{1,40} = -0.897$, $P = 0.375$).

Food provisioning at the end of the nestling stage

We found no significant difference in the feeding rate or food quantity delivered to carotenoid-supplemented or placebo fed young at the end of the nestling stage (male: feeding rate: $t_{1,39} = -0.264$, $P = 0.794$, food quantity: $t_{1,39} = 0.291$, $P = 0.773$, Fig. 2; female: feeding rate:

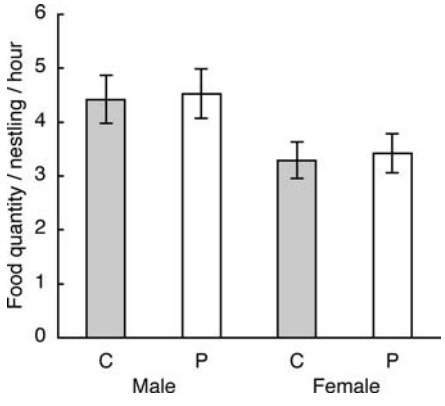

Fig. 1 Food provisioning of the male and female parent to carotenoid-supplemented (*C*) and placebo-fed (*P*) nestlings before feather appearance, i.e. 7 days post-hatching ($n = 37$ nests). Food quantity was calculated as the product of feeding rate and mean prey size (see Materials and methods). Means ± 1SE are shown

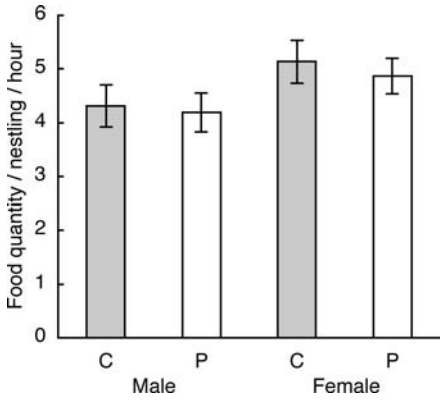

Fig. 2 Food provisioning of the male and female parent to carotenoid-supplemented (*C*) and placebo-fed (*P*) nestlings after feather appearance, i.e. 14 days post-hatching ($n = 43$ nests). Food quantity was calculated as the product of feeding rate and mean prey size (see Materials and methods). Means ± 1SE are shown

$t_{1,40} = -0.077$, $P = 0.939$, food quantity: $t_{1,\ 40} = 0.485$, $P = 0.630$, Fig. 2; total parents: feeding rate: $t_{1,\ 42} = -0.204$, $P = 0.840$, food quantity: $t_{1,\ 42} = 0.691$, $P = 0.494$), showing that neither the female nor the male preferentially fed the more colourful nestlings. Despite the considerable differences in plumage colouration, food provisioning to intensely coloured nestlings and control nestlings was similar (Fig. 2). The effect sizes ($d = 0.017$–0.201, $n = 43$) found in our study were small compared to Lyon et al. (1994) and with our effect size we would have needed a huge sample size ($n > 400$ broods) for finding any significant differences in the feeding behaviour to intensely coloured and control nestlings. Thus, the small differences in parental food provisioning between treatment groups are likely to be of small biological relevance.

Nestlings of the two treatment groups did not significantly differ in body mass (carotenoid-supplemented: 16.03 ± 0.154 g, placebo: 15.96 ± 0.151 g; $t_{1,\ 42} = 0.372$, $P = 0.712$) or metatarsus length (carotenoid-supplemented: 19.4 ± 0.06 mm, placebo: 19.4 ± 0.05 mm; $t_{1,\ 42} = 0.413$, $P = 0.682$) at the end of the nestling period. No significant interaction effect between the nestling rank 1 day after hatching and the carotenoid treatment on fledgling body mass was found (nested ANOVA: nestling rank × carotenoid treatment: $F_{1,\ 234} = 0.831$, $P = 0.368$).

Discussion

In the present study, we investigated the hypothesis that carotenoid-based nestling colouration has evolved in response to parental preference of intensely coloured offspring during food provisioning. Our experiment shows that, although nestling colouration could honestly signal offspring quality (Tschirren et al. 2003), parents did not discriminate between intensely coloured and control nestlings in their food provisioning behaviour at the end of the nestling stage. Similarly, nestling body mass and body size, both measures of the long-term consequences of a potential parental preference during food provisioning, did not significantly differ between carotenoid-supplemented and control nestlings. Our data thus suggest that it is unlikely that carotenoid-based colouration in nestling great tits has a signalling function in parent-offspring interactions during the nestling stage.

Further, we found no difference in the parental feeding behaviour to carotenoid-supplemented and control nestlings before feather appearance. Thus, our data do also not support the hypothesis that carotenoids might indirectly influence parental feeding decisions e.g. by colouring the mouth of the nestlings more conspicuous (Saino et al. 2000; Hunt et al. 2003), or by influencing the begging behaviour or sibling interactions.

Light is restricted in the nest box or natural nest holes where great tits rear their broods, and might constrain the perception of colour differences between offspring, even if the differences are as considerable as in our study (see e.g. Götmark and Ahlström 1997) for detection of mouth colour differences). Thus, carotenoid-based plumage colouration in juvenile great tits might not have evolved in parent-offspring interactions during the nestling stage but might be important after fledging.

After fledging juvenile great tits are tended for about 3 weeks by their parents (Gosler 1993), which provide them with food. Parents might selectively invest in high quality offspring during this period, especially when food is limited. Fledgling colouration is a reliable signal of offspring quality (Tschirren et al. 2003) upon which parents might base their feeding decisions (see Götmark and Olsson (1997) for parental feeding preference of artificially red painted great tit fledglings). Thus, carotenoid-based nestling colouration may have evolved in response to parental preference of intensely coloured young in food provisioning after fledging.

Alternatively, carotenoid-based colouration in fledglings may be important for establishing social hierarchies in foraging flocks that are formed by the birds after independence (i.e. status signalling hypothesis, Rohwer (1975), reviewed in Butcher and Rohwer (1989)). While the relationship between colour and dominance is well established for melanin-based colours (reviewed in Senar 1999) there is little evidence that carotenoid-based colours can act as signals of status in competitive interactions (e.g. Wolfenbarger 1999; McGraw and Hill 2000, but see Pryke et al. 2001). A potential signalling function of carotenoid-based colours in juvenile great tits for the establishment of dominance hierarchies thus needs further investigation.

On the other hand, carotenoid-based nestling colouration might have evolved in response to predation to which inexperienced juvenile birds are especially vulnerable after fledging (e.g. Gosler 1993). Although, the yellow breast plumage colouration appears conspicuous in the nest, it might provide good camouflage in the foliage of deciduous forests (background-matching

hypothesis, Baker and Parker 1979; Slagsvold and Lifjeld 1985) and thus reduce the predation risk.

Alternatively, the yellow breast plumage colouration of the fledglings may signal their escape ability or their general phenotypic quality towards a predator (unprofitable prey hypothesis, Baker and Parker 1979). Correlative (Johnsen et al. 2003) and experimental data (Tschirren et al. 2003) show that the expression of carotenoid-based colouration in nestling tits is condition-dependent i.e. birds in better condition have a more intense plumage colouration. Predators may thus preferentially hunt for pale individuals, which are easier to catch and may thereby select for the development of intense colouration in nestling birds.

As an alternative to the potential adaptive explanations for the evolution of carotenoid-based colours in juvenile birds, carotenoids might be passively deposited in the follicular cells of the developing feathers as a consequence of carotenoid ingestion with the food and have no special function. This scenario is plausible if pigmentation of feathers with carotenoids is associated with low costs. However, there is increasing evidence that the development of carotenoid-based colouration is costly (e.g. Hill and Montgomerie 1994; Hill 2000; Tschirren et al. 2003), supporting the idea that carotenoid-based colouration has beneficial effects for the juvenile birds.

In the present study, we found no evidence that intense yellow plumage colouration has a selective advantage for the juveniles during the nestling stage and there is no indication that carotenoids per se influence sibling interactions, begging, or parental food provisioning in the nest. Our study thus suggests that carotenoid-based colouration in nestling birds may have an adaptive function after fledging rather than in the nest.

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